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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Stable Transglutaminase Preparations and Processes for Producing Them

(72) Metzner, Hubert - Germany (Federal Republic of) ;
Karges, Hermann - Germany (Federal Republic of) ;

(71) BEHRINGWERKE AKTIENGESELLSCHAFT - Germany (Federal Republic of) ;

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BEHRINGWERKE AKTIENGESELLSCHAFT 1995/B006 - Ma 1015

Stable transglutaminase preparations and processes for producing them

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Abstract

The present invention relates to stable preparation forms of a transglutaminase, for example factor XIII, which, after lyophilization, are readily soluble without turbidity and which comprise the purified transglutaminase and also D- and/or L-amino acids, apart from glycine and arginine, their salts, derivatives and homologs, or dimers or oligomers thereof or mixtures thereof, and/or sugars or sugar alcohols, where appropriate in combination with surface-active agents and/or reducing agents. The invention also relates to processes for preparing stable protein preparations and to the use of the described stable preparation forms for producing pharmaceuticals which are suitable, for example, for treating diseases which are characterized by F XIII deficiency.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A stable preparation form of a transglutaminase which, after lyophilization, is readily soluble without any turbidity and which comprises the purified transglutaminase and also, as additives, D- and/or L-amino acids, their salts, derivatives and homologs, or dimers or oligomers thereof or mixtures thereof and/or sugars or sugar alcohols, where appropriate in combination with surface-active agents and/or reducing agents, with the exception of glycine or arginine.

2. The stable preparation form as claimed in claim 1, wherein the transglutaminase is factor XIII (F XIII), or biologically active fragments, derivatives or muteins thereof.

3. The stable preparation form as claimed in claim 2, wherein the F XIII is F XIII from plasma, placenta, thrombocytes or macrophages/monocytes or is rF XIII, or is biologically active fragments, derivatives or muteins thereof.

4. The stable preparation form as claimed in one of claims 1 to 3, wherein the amino acids are His, Glu, Met, Thr, Lys, Ala, Ile or Cys, or their salts, derivatives or homologs, or dimers or oligomers thereof, or mixtures thereof.

5. The stable preparation form as claimed in one of claims 1 to 4, wherein the sugar or sugar alcohol is sucrose, maltose, trehalose, lactose, sorbitol or mannitol, or their derivatives, homologs or mixtures.

6. The stable preparation form as claimed in claim 5,

wherein the sugars or sugar alcohols are present in combination with the amino acid His, Glu, Ile and/or Ala.

7. The stable preparation form as claimed in one of claims 1 to 6, wherein the surface-active agent is Tween 80, Tween 20, PEG, cetyl alcohol, PVP, PVA, lanolin alcohol or sorbitan monooleate.

8. The stable preparation form as claimed in one of claims 1 to 7, wherein the reducing agent is Cys, N-acetyl-Cys, thioglycerol, sodium sulfide or glutathione or mixtures thereof, where appropriate in the presence of a chelating agent.

9. The stable preparation form as claimed in one of claims 1 to 8 which comprises, as additives in addition to (an) amino acid(s), a sugar or sugar alcohol and a surface-active substance.

10. The stable preparation form as claimed in claim 9 which comprises, as additives, His/Tween 80/sucrose, His/Tween 20/sucrose, His/PEG/sucrose or His/Ile/PEG/sucrose.

11. The stable preparation form as claimed in one of claims 1 to 8, which comprises, as additives, (an) amino acid(s) and/or a sugar or sugar alcohol, a surface-active substance and, where appropriate, a reducing agent.

12. The stable preparation form as claimed in claim 11 which comprises, as additives, (an) amino acid(s)/Cys/ PEG/sucrose, (an) amino acid(s)/N-acetyl-Cys/PEG/ sucrose, (an) amino acid(s)/thioglycerol/PEG/sucrose or sugar/reducing agent/PEG, where appropriate in the presence of a chelating agent.

13. The stable preparation form as claimed in one of claims 1 to 12, wherein the concentration of the transglutaminase is in the range from 0.003 to 50 mg/ml.

14. The stable preparation form as claimed in one of claims 1 to 13, wherein the concentration of the amino acids, their salts, derivatives or homologs is in the range from 0.01 to 10% (w /v), preferably from 0.1 to 3% (w /v).

15. The stable preparation form as claimed in one of claims 1 to 14, wherein the concentration of the sugar or sugar alcohol is between 0.1 and 20% (w / v), preferably between 0.2 and 10% (w /v).

16. The stable preparation form as claimed in one of claims 1 to 15, wherein the concentration of the surface-active agent is between 0.00001 and 5% (w / v), preferably between 0.0002 and 0.1% (w /v).

17. The stable preparation form as claimed in one of claims 1 to 16, wherein the concentration of the reducing agent is between 0.001% and 2% (w /v), preferably between 0.005 and 0.5% (w /v).

18. The stable preparation form as claimed in one of claims 1 to 17, wherein the pH is in a range from 6 to 9, preferably between 7 and 8.

19. The stable preparation form as claimed in claims 1 to 17 which comprises a borate buffer having a pH in the range from 6 to 9 and, where appropriate, a chelating agent.

20. The stable preparation form as claimed in claims 1 to 17 which comprises a Tris buffer having a pH in the range from 6 to 9 and, where appropriate, a chelating agent.

21. The use of one or more stabilizing additives as claimed in one of claims 1 to 20 for preparing a stable liquid protein preparation.

22. A process for preparing a stable protein preparation, which comprises mixing the purified protein(s), as a solution or precipitate, with a solution which contains one or more additives as claimed in one of claims 1 to 20, and freeze-drying.

23. The process as claimed in claim 22, wherein the protein is a transglutaminase.

24. The use of the transglutaminase which has been stabilized in accordance with the process of claim 23 for preparing a pharmaceutical.

25. The use of the stable preparation form of a transglutaminase as claimed in one of claims 1 to 20 for preparing a pharmaceutical for treating diseases which are characterized by F XIII deficiency.

26. The use of the stable preparation form of a transglutaminase as claimed in one of claims 1 to 20 for preparing a pharmaceutical for treating diseases which can be positively influenced by the topical or parenteral administration of this transglutaminase.

BEHRINGWERKE AKTIENGESELLSCHAFT

1995/B006 - Ma 1015
Dr. Lp./Mi

Stable transglutaminase preparations and processes for producing them

The present invention relates to stable transglutaminase preparations, in particular stable factor XIII preparations, and processes for producing them.

Factor XIII (F XIII, fibrin-stabilizing factor), which is a transglutaminase which is present as a proenzyme in plasma, platelets and monocytes/macrophages, is of importance, inter alia, for ensuring efficient blood coagulation and wound healing. F XIII, in the form of fresh frozen plasma, isolated from placenta or plasma, has already been successfully employed therapeutically for many years for the therapy of factor XIII deficiency. In the meantime, it has also become possible to prepare factor XIII recombinantly (rF XIII).

The commercially available, purified or partially purified transglutaminase or F XIII preparations contain added stabilizers, such as human serum albumin (HSA), which is, however, a disadvantage for protein preparations in view of the concomitant decrease in the specific activity, the additional protein load and/or potential immunogenicity and also the adverse effect on assessment of purity. Particularly in the case of highly pure proteins (such as recombinant proteins), it is desirable not to reduce once again the purity which has been achieved by adding foreign proteins. Furthermore, there is the potential for contamination with viral antigens when albumin, for example, is added.

The composition and activity of protein preparations which can be employed therapeutically generally must be stable over a relatively long period of time. Since it

is only rarely possible to achieve this in solution, such products are frequently marketed in the dry state. Mild freeze-drying is the method of choice for drying such products. However, even when this method is used, stable preparations, which fulfill the requirements for integrity and durability, are only obtained under certain conditions.

Since freeze-drying unstabilized transglutaminase solutions, for example, leads to a marked drop in activity and to considerable turbidity, formulations based on albumin and containing relatively high concentrations of salts have, for example, been previously described for use with purified F XIII preparations (DE-C-2063 070, JP 53/59018). However, these formulations suffer from the previously described disadvantage of the addition of foreign protein, with all the problems attached thereto.

The freeze-drying of rF XIII in the presence of glycine or arginine and non-reducing sugars has also been described (WO 93/03147). In this case, however, no statements were made with regard to the stability and solubility or clarity of the reconstituted lyophilisate. Furthermore, the resulting product was stored at -20°C, presumably to keep the material stable owing to inadequate stability at 4°C.

The underlying object of the present invention is, therefore, to obtain, by means of a suitable formulation for transglutaminases, in particular for F XIII, a non-perishable product, as a protein which can be administered locally (e.g. topically as well) or parenterally, which is stable at 2-8°C (or higher) and which does not require the addition of, for example, HSA. Furthermore, the lyophilisate should be readily soluble and, after having been dissolved, should yield a clear solution which lacks turbidity and which, in addition, should also be sufficiently stable.

This objectivewas achieved by the provision of stable preparation forms for transglutaminase preparations, obtained by using defined stabilizers or mixtures thereof, as described in more detail below, and by processes for producing them. The invention consequently relates to a stable preparation form of a transglutaminase which, after lyophilization, is readily soluble without turbidity and which comprises the purified transglutaminase and also D- and/or L-amino acids, apart from glycine and arginine, their salts, derivatives and homologs, or dimers or oligomers thereof or mixtures thereof and/or sugars or sugar alcohols, where appropriate in combination with surface-active agents and/or reducing agents.

While the exemplary embodiments demonstrate this with reference to recombinant factor XIII or F XIII which is isolated from placenta or plasma, they are not restricted thereto. In a preferred embodiment, the present invention consequently relates to stable preparation forms of factor XIII, and to biologically active fragments, derivatives or muteins thereof.

In a particularly preferred embodiment, the stable preparation forms are preparation forms which comprise F XIII from plasma, placenta, thrombocytes or macrophages/monocytes, or comprise recombinant F XIII.

The novel investigations can essentially be divided into two areas: (a) investigations on the freeze drying itself, and (b) on subsequent stability during storage.

rF XIII (Metzner et al., in J. McDonagh, R. Seitz, R. Egbring: "Factor XIII", 87 - 93, Schattauer (1993)) and also placental and plasma F XIII (Karges and Rapp, in J. McDonagh, R. Seitz, R. Egbring: "Factor XIII", 66 - 76, Schattauer (1993)) were employed in the investigations.

The freeze-drying experiments were carried out in commercially available units using small glass bottles with and without a siliconized surface.

(a) Freeze-drying:

In a comparison of different additives, surprisingly it was found, that the activity and solubility of the F XIII can be preserved very well during freeze-drying in some cases when certain additives from the group of the D- and/or L-amino acids, their salts, derivatives or homologs are used (Table I).

In a further preferred embodiment, the present invention relates, therefore, to stable preparation forms of a transglutaminase which comprise the amino acids His, Glu, Met, Thr, Lys, Ala, Ile or Cys, their salts, derivatives or homologs, dimers or oligomers thereof, or mixtures thereof.

The amino acids His and Glu, especially, surprisingly exhibited good stabilization during freeze-drying, even without any further additives. On the other hand, when amino acids such as Gly, Met or Ala were used on their own, there was a perceptible decline in activity during the freeze-drying (Table I).

In some cases, the use of sugars or sugar alcohols on their own afforded good stabilization during the course of the freeze-drying (Table I). In particular, sugars or sugar alcohols such as sucrose, trehalose, lactose, maltose, sorbitol, mannitol, or the like, gave positive results. The decline in activity which takes place during freeze-drying when amino acids are added which were not sufficiently effective on their own (such as Met, Ala, inter alia) can be markedly reduced by combining the amino acids with sugars or sugar alcohols (Table I).

In a further embodiment, the present invention relates, therefore, to stable preparation forms of a transglutaminase which comprise the sugars or sugar alcohols sucrose, lactose, trehalose, maltose, sorbitol or mannitol, their derivatives, homologs or mixtures thereof.

Only negligible stabilization was achieved when buffering substances such as Tris or phosphate were employed on their own. However, the use of borates resulted in perceptible stabilization during freeze-drying (Table I).

A slight protein precipitate which appeared on some occasions when the lyophilisate was dissolved was averted by using surface-active substances such as Tween 80 or Tween 20, polyethylene glycol (PEG) of molecular weights between 1000 and 35000 Da, cetyl alcohol, polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), lanolin alcohol, sorbitan monooleate, inter alia, without the F XIII losing activity during the freeze-drying (Tables II and III).

In a further preferred embodiment, the stable preparation forms consequently comprise, as a surface-active substance, Tween 80, Tween 20, PEG, cetyl alcohol, PVP, PVA, lanolin alcohol or sorbitan monooleate.

(b) Stability during storage:

A critical parameter of the formulated lyophilisate is its storage stability, which was determined at 4°C and at room temperature, and also, under accelerated conditions, at 37°C.

It was found that the combination of amino acids and sugars, sugar alcohols or sugar derivatives was advantageous for good storage stability (Table IV). Investigation using different sugars showed that

while sugars such as sucrose, lactose, trehalose or maltose stabilize activity even during relatively long storageperiodsat elevated temperature, sugars such as glucose or fructose, as reducing sugars, are unable, to a sufficient extent, to prevent a slow decline in activity at 37°C (Table V). Combining the amino acids His or Glu, which give rise to good stability even on their own, with sugars also resulted in a stability which was still further elevated.

In a further preferred embodiment, the stable preparation forms consequently comprise, as stabilizer, sucrose, maltose, trehalose, lactose, sorbitol or mannitol, or their derivatives or homologs, or mixtures thereof, in combination with the amino acid His, Glu, Ile and/or Ala.

As has already been mentioned, surface-active substances had no negative effects on storage stability when used in the appropriate concentration range (Tables II and III).

Although F XIII does not possess any accessible SH groups, SH agents such as Cys, N-acetylcysteine, thioglycerol or glutathione surprisingly exhibit a positive effect on the storage stability of F XIII, especially at elevated temperatures. In this connection, chelating agents such as EDTA or citrate can be added to protect the SH functions.

In a further preferred embodiment, the novel, stable preparation form of a transglutaminase therefore comprises Cys, N-acetyl-Cys, thioglycerol, sodium sulfide or glutathione, or mixtures thereof, where appropriate in the presence of a chelating agent.

Very good results as regards maintaining the activity and the solubility of the lyophilisate were

achieved using ternary or quaternary mixtures (amino acid(s), sugar and surface-active component), for example using His/Tween/sucrose mixtures, His/PEG/sucrose mixtures or His/Ile/PEG/sucrose mixtures. In a further preferred embodiment, the novel, stable preparation form of a transglutaminase consequently comprises as additives, apart from (an) amino acid(s), a sugar or sugar alcohol and a surface-active substance, with the additive mixtures His/Tween 20/sucrose or His/Tween 80/sucrose, His/PEG/sucrose or His/Ile/PEG/sucrose being particularly preferred in this context.

With regard to stablity during storage, mixtures composed of (an) amino acid(s) and/or sugar or sugar alcohol, a surface-active substance and a reducing agent, e.g. mixtures composed of amino acid(s)/Cys or N-acetyl-Cys/PEG/sucrose, amino acid(s)/thioglycerol/ PEG/sucrose and sugar/reducing agent/PEG likewise proved to be suitable formulation systems, especially at higher temperatures as well (see Tables III and VI). In a further preferred embodiment, the novel preparation form therefore comprises, as additive, a mixture composed of (an) amino acid(s) and/ or sugar or sugar alcohol, a surface-active substance and a reducing agent, with the mixtures amino acid(s)/Cys/PEG/sucrose, amino acid(s)/N-acetyl-Cys/PEG/sucrose, amino acid(s)/thioglycerol/PEG/ sucrose and sugar/reducing agent/PEG, where appropriate in the presence of a chelating agent, being particularly preferred.

In the described formulations, the concentration of F XIII which is employed can be varied in a wide range and is preferably in the range of 0.003-50 mg/ml.

The concentrations of the amino acids which are employed are preferably in a range of 0.01-10% (w /

v), particularly, however, in a range of 0.1-3% (w /v). The sugar concentrations are preferably 0.1-20% (w /v), particularly preferably, however, between 0.2 and 10% (w /v). Surface-active components may be employed in a preferred concentration range of 0.00001-5% (w /v), particularly preferably between 0.0002% and 0.1%. The concentrations of the reducing agents are preferably between 0.001% and 2% (w /v), particularly, however, between 0.005% and 0.5%.

The residual moisture is also of importance for the stability of the lyophilized protein during storage. Using the given additives, the temperatures can be raised to 50-60°C for several hours, insofar as this is necessary for reducing the residual moisture, during a final phase of the freeze-drying, without any loss of activity.

For the purpose of freeze-drying transglutaminases, or F XIII, and also for their subsequent stability during storage, the pH of the solutions should preferably be in the range of 6-9, particularly preferably between 7 and 8. The described amino acids or phosphate buffers, borate buffers or Tris buffers having a pH in the range of 6 to 9, which are used, where appropriate, in combination with a chelating agent, are preferred for effecting the buffering.

The present invention also encompasses the use of the above-described stabilization additives for preparing stable liquid preparations which comprise (a) protein(s), since the results obtained from the experiments carried out on transglutaminases, by way of example, are applicable to other preparations.

The present invention furthermore encompasses a process for stabilizing proteins, preferably trans-

glutaminases, in which the purified proteins or the purified protein, which is preferably a transglutaminase, are/is mixed, in accordance with the customary procedure, as a solution or precipitate, with a solution which contains one or more of the novel additives and is adjusted to a pH range which is advantageous for stability, after which the whole is freeze-dried.

Due to the outstanding properties of the stabilized transglutaminases which are prepared in accordance with the novel process, these transglutaminases are very well suited for formulating a pharmaceutical.

The present invention also includes the use of stable preparation forms which comprise factor XIII, or biologically active fragments, derivatives or muteins thereof, for preparing a pharmaceutical for treating, for example, diseases which are characterized by F XIII deficiency.

Where appropriate, the novel pharmaceuticals can be formulated, in accordance with known methods, with suitable, pharmaceutically tolerated, well-known excipients. They can be administered in an appropriate dose which can be determined by the doctor in attendance. Administration can be effected by a variety of routes, for example intravenously, intraperitoneally, subcutaneously, intramuscularly, intradermally or topically.

The examples illustrate the invention.

Example 1

rF XIII, which was prepared by expression in yeast cells and purified to a purity of >98% by means of suitable methods, and also placental F XIII, likewise in purified form, were treated, as a solution or as a precipitate, with solutions of the stabilizers in order to attain the given activities. The F XIII solutions were filtered, used to fill small glass bottles and dried in accordance with a suitable freeze-drying program. The lyophilisates were then reconstituted once again to the original volume using distilled water. The F XIII activities were determined before and after the freeze-drying. In addition, the reconstituted solution was assessed with regard to turbidity.

The activity of the F XIII was determined using the commercially available Berichrom F XIII^{R} test kit.

The results presented in Tables I, II and III demonstrate unambiguously that it is necessary to add the novel stabilizing constituents when freeze-drying F XIII and that the enzymic activity and the solubility can be preserved by adding amino acids or sugars or amino acids and sugars even without the additional use of HSA. The solubility, in particular, can in some cases be still further improved by adding surface-active agents.

Example 2

Lyophilisates of rF XIII and placental F XIII, which had been prepared as indicated in Example 1, were stored at 4°C or at 37°C and reconstituted with Aqua Injectab. or with sodium chloride solution after various times in order to determine the enzyme activity which remained.

The results presented in Tables II to VI demonstrate that adequate long-term stabilization can be achieved using the already described mixtures comprising an amino acid or amino acids and/or sugars or sugar derivatives. The

addition of a reducing component leads in some cases to a further increase in stability, particularly under accelerated conditions.

Table I: Freeze-drying FXIII

	Activity before lyophilis. (%)	Activity of the lyophilisate (in % of the starting value)	Turbidity after dissolving the lyophilisate
-	100	2	+++
10 mM Tris* HCl, pH 7.4	100	8	+
10 mM Na borate pH 8.0	100	68	(-)
1% L-His, phys. NaCl, pH 7.4	100	39	++
1% L-His, pH 7.6	100	100	-
1% L-Arg, pH 7.6	100	96	+/-
1% L-Gly, pH 7.6	100	41	+/-
1% L-Ala, pH 7.6	100	67	+/-
1% L-Glu, pH 7.6	100	88	+
1% L-Met, pH 7.6	100	10	+++
1% L-His, 0.1% L-Ile, pH 7.6	100	99	-
1% sucrose	100	92	(-)
2.5% sucrose	100	91	(-)
5% sucrose	100	83	(-)
2.5% lactose	100	100	(-)
2.5% sorbitol	100	92	(-)
2.5% trehalose	100	99	(-)
2.5% maltose	100	100	(-)
1% L-His, 2.5% sucr., pH 7.6	100	96	(-)
1% L-Arg, 2.5% sucr., pH 7.6	100	90	+

Continuation: Table 1

	Activity before lyophilis. (%)	Activity of the lyophilisate (in % of the starting value)	Turbidity after dissolving the lyophilisate
1% L-Ala, 2.5% sucr., pH 7.6	100	100	-
1% L-Glu, 2.5% sucr., pH 7.6	100	87	+
1% L-Lys, 2.5% sucr., pH 7.6	100	92	(-)
1% L-Met, 2.5% sucr., pH 7.6	100	91	(-)
1% L-Thr, 2.5% sucr., pH 7.6	100	94	+/-
1% L-His, 0.1% L-Ile, 2.5% sucr., pH 7.6	100	94	(-)
1% L-His, 0.1% L-Ile, 2.5% gluc., pH 7.6	100	85	-
1% L-His, 0.1% L-Ile, 2.5% lact., pH 7.6	100	99	(-)
1% L-His, 0.1% L-Ile, 2.5% fruct., pH 7.6	100	92	-
1% L-His, 0.1% L-Ile, 2.5% sorbitol, pH 7.6	100	91	(-)
0.5% L-His, 0.1% L-Ile, 2.5% sucr., pH 7.6	100	95	(-)
1% L-His, 0.1% L-Ile, 1% sucr., pH 7.6	100	93	(-)
1% L-His, 0.1% L-Cys, 2.5% sucr., pH 7.6	100	107	-

Activity test: Berichrom FXIII

Turbidity: - no turbidity
(-)minimal turbidity
+/-very low turbidity
+slight, but very distinct, turbidity
++marked turbidity
+++very marked turbidity

Table II: Effect of surface-active substances on the solubility or stability of an FXIII lyophilisate

	Activity before lyophilization (U/ml)	Turbidity after dissolving	Activity (U/ml) at t =							
			0	0.5	1	2	3	6	12	24 mon.
1% L-His, 0.1% L-Ile, 2.5% sucr., pH 7.2	153	+	150	144	139		139	143	136	
1% L-His, 0.1% L-Ile, 2.5% sucr., pH 7.6	154	+	141	151	132		132	139	136	
1% L-His, 0.1% L-Ile, 2.5% sucr., pH 8.0	152	+	148	143	141		141	138	137	
1% L-His, 0.1% L-Ile, 2.5% sucr.	129	+	124		122	113	115	111	115	122
1% L-His, 2.5% sucr.	131	(-)	125		126	114	117	108	118	120
1% L-His, 0.01% PEG 4000, 2.5% sucr.	128	-	119		124	114	111	111	114	119
1% L-His, 0.001% PEG 4000, 2.5% sucr.	130	-	123		125	112	114	112	114	119
1% L-His, 0.0001% PEG 4000, 2.5% sucr.	130	-	118		120	107	114	107	107	116
1% L-His, 0.001% Tween 20, 2.5% sucr.	128	-	123		124	112	114	113	119	119
1% L-His, 0.0001% Tween 20, 2.5% sucr.	130	-	122		124	112	111	105	112	120
1% L-His, 10mM citrate, 2.5% sucr.	156	(-)	149	146	138		143	136	147	
1% L-His, 0.01% PEG 4000, 2.5% sucr.	155	-	147	138	137		140	134	135	
1% L-His, 0.1% L-Cys, 2.5% sucr.	156	-	156	156	146		148	153	151	

Storage of the samples at +4°C pH 7.6, unless otherwise indicated

Activity test: Berichrom FXIII (U/ml)

Turbidity: - no turbidity (-) minimal turbidity + slight, but very distinct, turbidity

Table III: Storage of FXIII lyophilisate

Storage of the samples at room temperature

	Activity before storage (U/ml)	Activity (U/ml) at t =					Turbidity after t =
		1 mon. (U/ ml)	3 mon. (U/ ml)	6 mon. (U/ ml)	9 mon. (U/ ml)	12 mon. (U/ ml)	1 mon.
1% His/0.001% PEG 4000/2.5% sucr./pH 7.6	85	99	89	84	86	69	(-)
1% His/0.1% cysteine/0.001% PEG 4000/2.5% sucr./pH 7.6	92	110	97	103	110	91	-
1% His/0.01% cysteine/0.001% PEG 4000/2.5% sucr./pH 7.6	87	108	96	98	104	85	-
1% His/0.005% PEG 4000/2.5% sucr./pH 7.6	99	109	103	99	94	79	-
1% His/0.01% PVP 15/2.5% sucr./pH 7.6	94	105	96	96	88	78	-
1% His/0.1% Ile/ 0.001-% PEG 4000/ 2.5% sucr./ pH 7.6	101	106	92	84	90	69	-

Activity test: Berichrom FXIII

Turbidity: - no turbidity (-) minimal turbidity

Table IV: Storage of rhuFXIII lyophilisate at 4°C

Activity before lyophilisation (U/ml)	Activity (U/ml) of the lyophilisate at t =							
	0	0.5 mon	1 mon	3 mon	6 mon	12 mon	18 mon	24 mon
1% L-His pH 7.6	125	150	112	95	111	82	100	108	100
1% L-Gly pH 7.6	125	61	26	48	34	30	29	25	16
1% L-Glu pH 7.6	125	132	127	116	110	77	114	83	93
1% L-His, 2.5% sucr., pH 7.6	125	144	112	106	133	97	111	109	94
1% L-Arg, 2.5% sucr., pH 7.6	125	134	113	106	118	88	113	60	89
1% L-Ala, 2.5% sucr., pH 7.6	125	150	124	98	114	71	127	102	118
1% L-Glu, 2.5% sucr., pH 7.6	125	130	108	110	122	84	140	104	90
1% sucrose	160	153	130	139	136	137	141	129	146
2.5% sucrose	160	148	127	135	128	135	130	126	141
5% sucrose	160	130	109	117	113	109	114	107	121
2.5% lactose	160	163	136	146	137	137	137	131	158
2.5% sorbitol	160	146	122	132	125	122	123	124	148
2.5% trehal-ose	160	160	136	142	137	141	136	127	138
2.5% maltose	160	155	134	139	132	118	139	123	150
1% L-His, 0.1% L-Ile, pH 7.6	125	148	136	126	93	100	116	105	102
0.5% L-His, 0.1% L-Ile, 2.5% sucr., pH 7.6	125	142	123	113	119	93	116	106	100
1% L-His, 0.1% L-Ile, 1% sucr., pH 7.6	125	139	112	109	127	94	87	108	103
1% L-His, 0.1% L-Cys, 2.5% sucr., pH 7.6	125	161	135	120	113	91	112	112	101

Activity test: Berichrom FXIII

Table V: Storage of FXIII lyophilisate at 37°C

	Activity before lyophil. (U/ml)	Activity (U/ml) of the lyophilisate at t =				
		0	1 mon	3 mon	6 mon	12 mon
1% L-His, 0.1% L-Ile, 2.5% sucr., pH 7.6	444	394	405	311	300	212
1% L-His, 0.1% L-Ile, 2.5% glucose pH 7.6	440	373	358	152	34	3
1% L-His, 0.1% L-Ile, 2.5% lactose pH 7.6	424	419	385	322	293	285
1% L-His, 0.1% L-Ile, 2.5% fructose, pH 7.6	423	387	385	37	11	3
1% L-His, 0.1% L-Ile, 2.5% sorbitol pH 7.6	420	380	298	120	64	21
1% L-His, 0.1% L-Ile, 2.5% maltose pH 7.6	425	366	393	316	293	293
1% L-His, 0.1% L-Ile, 0.1% L-Cys, 2.5% sucrose, pH 7.6	413	408	418	350	354	393
1% L-Glu, 0.1% L-Ile, 2.5% sucr., pH 7.6	424	395	350	281	301	314
1% L-Lys, 0.1% L-Ile, 2.5% sucr., pH 7.6	423	350	403	328	318	333

Activity test: Berichrom FXIII

Table VI: Storage of FXIII lyophilisate at various temperatures

	Activity before lyophil. (U/ml)	Activity (U/ml) after various storage times at +4°C in months						
		0	0.5 mon	1 mon	3 mon	7 mon	12 mon	24 mon
1% His/ 0.001% PEG/2.5% cysteine/ sucr./0.2% pH 7.6	112	118	119	117	116	115	122	120
1% His/ 0.001% PEG/2.5% sucr./ 0.2% N-acetyl-cysteine/ pH 7.6	112	113	123	120	125	118	120	125
1% His/ 0.001% PEG/2.5% sucr./ 0.2% thioglycerol/ pH 7.6	119	109	125	122	129	116	129	131
1% His/ 0.001% PEG/2.5% sucr./ pH 7.6	119	109	117	110	118	109	113	116

Storage at room temperature

		0	0.5 mon	1 mon	3 mon	7 mon	12 mon	24 mon
1% His/0.001% PEG/2.5% sucr./0.2% cysteine/ pH 7.6	112	118	119	115	116	114	120	112
1% His/0.001% PEG/2.5% sucr./0.2% N-acetylcysteine/pH 7.6	112	113	112	114	120	122	122	120
1% His/0.001% PEG/2.5% sucr./0.2% thioglycerol/pH 7.6	119	109	123	121	120	116	118	116
1% His/0.001% PEG/2.5% sucr./pH 7.6	119	109	117	108	109	105	100	98

Continuation of Table VI: Storage of FXIII lyophilisate at various temperatures

Storage at +37°C

		0	0.5 mon	1 mon	3 mon	7 mon	12 mon	24 mon
1% His/0.001% PEG/2.5% sucr./0.2% cysteine/ pH 7.6	112	118	121	114	119	119	104	89
1% His/0.001% PEG/ 2.5% sucr./0.2% N-acetylcysteine/pH 7.6	112	113	119	111	112	102	107	89
1% His/0.001% PEG/2.5% sucr./0.2% thioglycerol/pH 7.6	119	109	119	120	113	95	98	85
1% His/0.001% PEG/2.5% sucr./pH 7.6	119	109	107	108	100	85	78	57

Activity test: Berichrom FXIII